Chronic repetitive transcranial magnetic stimulation-induced increases in GABAergic neurotransmission in chronic unpredictable mild stress rat model: 1H-NMR spectroscopy study at 11.7 T

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INTRODUCTION

Repetitive transcranial magnetic stimulation (rTMS) that use magnetic fields to stimulate focal cortical regions with electrical current has been used for evolving treatment for refractory depression. Despite of its broad use, little information about neither the precise pattern of brain activation nor the molecular mechanisms underlying rTMS effects are known. Recent studies suggest that the serotonergic involvement in depression may linked to the action of GABA [1]. Deficits in GABA-containing neurons are consistently reported in psychiatric disease, particularly in the frontal cortex and hippocampus [2]. Thus, GABA might be potential candidate for rTMS-induced changes on the central nervous system. To investigate this hypothesis, we established an animal model of depression using chronic unpredictable mild stress (CUMS), which the procedure results in a number of behavioral abnormalities that can be seen in patients with depression. Then, the effects of rTMS on GABA in rat brain were assessed using ^{1}H-NMR spectroscopy technique.

MATERIALS AND METHODS:

Animals Male Sprague-Dawley rats (180 ± 20 g, N=20) were used and randomly divided into four groups: control + sham (N=5), control + rTMS (N=5), CUMS + sham (N=5), and CUMS + rTMS (N=5). During whole experimental procedures, rats were housed in single cages. For 4 weeks, the rats for stressed groups were subjected to a weekly regimen of mild stress [3] (Table 1). And sucrose intake (1 % sucrose solution) and body weight were measured once a week during 1 h window of food and water deprivation. Baseline was measured less than 1 week before the start of CUMS.

Table 1. Schedule of chronic unpredictable mild stress

Mon.	Confinement (10:00-13:00), Food and water deprivation (10:00-next day 11:00), Soiled cages (15:00-next day 09:00)
Tues.	Cage cleaning and body weighting recording (09:00), Sucrose preference test (10:00-11:00), Restricted access to food (11:00-13:00), Confinement (15:00-18:00), Cage tilt (18:00-next day 09:00)
Wed.	Group housing (N=5, 09:00-17:00), Empty bottle (09:00-11:00), Food and water deprivation / Foreign object in cage (17:00 - next day 10:00)
Thurs.	Cage tilt (10:00-17:00), Continuous lighting (17:00 - next day 10:00)
Fri.	Restricted access to food (10:00-12:00), Forced swimming test for 5 min (14:00), Confinement (15:00-19:00), Soiled cage (19:00 - next day 10:00)
Sat.	Food and water deprivation (10:00-next day 09:00), Foreign object in cage (17:00 - next day 12:00)
Sun.	Group housing (17:00 - next day 10:00)

Chronic rTMS treatment Rats were treated with rTMS for 4 weeks with alternative day. After the termination of CUMS procedures, rTMS was delivered to rats using a Neopulse stimulator (Neotonus Inc., Marietta, USA) and a 7 cm diameter figure-8 coil. Stimulation was delivered at a rate of 10 Hz for 10 min. The intensity of the stimulation was 1.4 T at the surface of the coil. The stimulation consisted of 20 trains of 50 pulses with a 25-sec pause between each successive train. The sham group was only exposed to the acoustic artifact of rTMS without the stimulation itself. ***^{1}H-NMR spectroscopy*** The prefrontal cortex and hippocampus tissues were harvested from rat brain. The brain tissues ground to a find powder before the extract solvents were added. The metabolites were extracted using methanol-chloroform-water (MCW) method as previously described [4]. All dried extract samples (N=40) were dissolved in D_2O containing TSP (0.05%) with volume of 500 ul and adjusted to pH 6.9 -7.1. The NMR spectroscopic data were acquired using CPMG pulse sequence (16010 complex points, 8000 Hz spectral window, 2-s presat delay 2-s acquisition time). The metabolites were quantified using LCModel. ***Statistical analysis*** The PASW software was used for two-way ANOVA test (brain region × treatment).

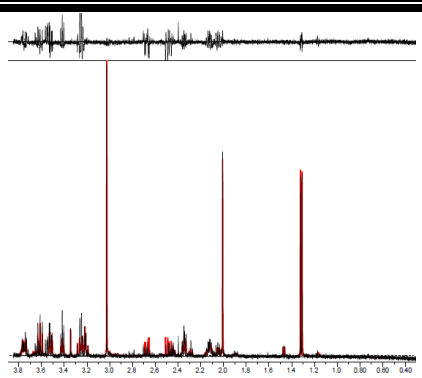

Fig.1. Representative ^{1}H-NMR spectra of prefrontal cortex of rat is shown. The spectra was fitted with LCModel.

RESULTS AND DISCUSSION

The representative ^{1}H-NMR spectra of MCW-extracted sample is shown in Fig.1. Two-way ANOVA test revealed that there were significant differences in GABA concentrations among four groups (post hoc test--> Prefrontal Cortex: control + rTMS vs. CUMS + sham, p=0.001, control + rTMS vs. CUMS + rTMS, p=0.003; Hippocampus: control + rTMS vs. CMS + sham, p=0.003), which can be seen in Fig. 2. The chronic rTMS effects were more pronounced in prefrontal cortex because of the proximity of this region.

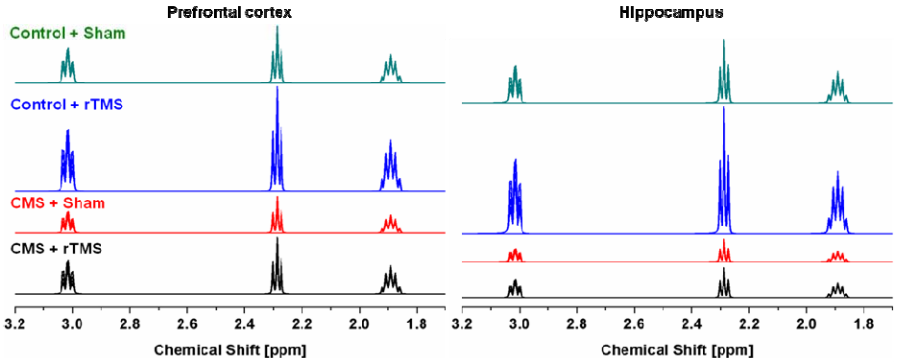

Fig.2. Average GABA spectra of each group from LCModel fit. The spectra for each group are shown with the same scale. Note that chronic rTMS enhance GABA contents in both control and stressed group, particularly in prefrontal cortex.

CONCLUSION

Our finding indicates that chronic rTMS has a modulatory effect on GABAergic systems, suggesting its beneficial effects.

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REFERENCES: [1] Slattery DA, et al., J Pharmacol Exp Ther (2005) [2] Reynolds GP et al., Biochem Soc Trans (2007) [3] Willner P et al. Psychopharmacology (1987) [4] Le Belle et al. NMR Biomed (2002)